



**Figure 1 -- figure supplement 2. Characterization of Eed<sup>CKO</sup> mice.**

- A.** Progressive cardiac dysfunction and dilatation after cardiomyocyte-restricted ablation of Eed. w, weeks.
- B.** *Nppa* mRNA level in WT and CKO hearts at the indicated ages. w, weeks.
- C, D.** Cardiac fibrosis was evident by Mason Trichrome staining at 2 months of age. Fraction of myocardial area occupied by fibrotic tissue (blue staining) was quantified using ImageJ. Bar = 50  $\mu$ m.
- E, F.** Immunofluorescence for cardiomyocyte marker TNNI3 and cardiomyocyte membrane marker WGA, and quantification of cell size from WGA-stained cardiomyocyte outlines (f). Bar = 50  $\mu$ m.
- G.** Immunostaining for TNNI3 and H3K27me3. Isolated adult cardiomyocytes were > 95% pure and Eed<sup>CKO</sup> CMs had little H3K27me3 signal. Bar = 50  $\mu$ m.
- H.** PCR of genomic DNA from purified CMs using primers that amplify unexcised floxed DNA (233 bp product) or Cre-excised DNA (453 bp product). In CKO-purified CMs, unexcised floxed DNA was not detected, consistent with highly efficient Cre-mediated gene inactivation, as well as high purity of dissociated CMs.
- I.** RNA-seq track view showing deletion of floxed exons 3-6 of *Eed* (red box).
- J, K.** Genome browser view of H3K27me3 and H3K27ac ChIP-seq signals on *Myh6* (J) and *Vim* (K) loci in purified adult cardiomyocytes.
- L.** EED enrichment on downstream genes was measured by ChIP-qPCR in P5 heart ventricle apex. Numbers following gene names indicate the number of nucleotides between the probed amplicon and the TSS.
- M.** Box and scatter plots of H3K27me3 at TSS  $\pm$  500 bp of EED target genes in four quantiles of WT H3K27me3 intensity.
- N.** Aggregation plots of H3K27me3 ChIP-seq signals near the TSS of genes upregulated, downregulated, or unchanged between WT and EED<sup>CKO</sup>.
- O.** H3K27me3 enrichment was measured by ChIP-qPCR on target genes using adult cardiomyocytes isolated from WT and EED<sup>CKO</sup> hearts.
- \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  by ANOVA with Dunnett's post-hoc test using Eed<sup>fl/+</sup>::Myh6-Cre<sup>-</sup> as the control group (A), by Welch's *t*-test (B, D, F, N, O), or by Wilcoxon-Mann-Whitney test (M).